8th ISMB Symposium 18-19 June 2018

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The Institute of Structural and Molecular Biology holds a varied programme of events for its researchers and students throughout the academic year. ISMB symposia, held in 'even years', feature talks from international research leaders; in retreats, held in 'odd years', the emphasis is on the work of the Institute's students and postdocs. The 2018 symposium was the eighth in the series and, unusually, it took place over two half days: June 18 and 19. The lecture hall in Birkbeck's Clore Management Centre was full to capacity throughout. This was also the first symposium to take place at the same time as Birkbeck's annual Science Week, and the first to include the college's Rosalind Franklin lecture.

In introducing the symposium, Gabriel Waksman, the ISMB's 'indefatigable' founder director, explained that the ISMB had three things to celebrate at this symposium. It was fifteen years since the Institute was founded, its new website at http://www.ismb.lon.ac.uk was finally live, and a new electron microscope had recently been installed and switched on. With this state-of-the-art microscope – a Titan Krios, suitable for both single particle analysis and cryo-tomograpy of larger systems – in place, Institute scientists are now even better placed to take advantage of the so-called 'resolution revolution' in this technique. Not surprisingly, electron microscopy was to be a key focus of the symposium. Waksman also introduced a live tweeting competition for PhD students, who were encouraged to tweet using the hashtag #ISMBLondon2018; there would be prizes for the posters of the wittiest or most original tweets. He warned student tweeters not to 'be obnoxious' and not to inadvertently 'publish' unpublished work.

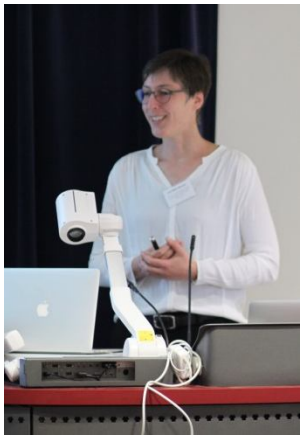
After a few words from Helen Saibil FRS, a world-leading electron microscopist who had pioneered the technique at Birkbeck in the 1980s, the symposium was formally opened by the dean of life sciences at UCL, Geraint Rees. He praised Waksman for leading a 'deep strategic partnership' between two very different research institutions, and handed over to the session chair to introduce the first speaker, Professor Sir Tom Blundell.

Blundell, now an emeritus professor at Cambridge University, is no stranger to Bloomsbury: he was appointed as Professor of Crystallography at Birkbeck in succession to J D Bernal in 1976 and made head of department two years later. His engaging talk covered a wide range of topics in structural and chemical biology 'from Bernal to Birkbeck and beyond'. He started close to the end by introducing Astex, the drug discovery company that he founded in the late 90s with Harren Jhoti, one of his PhD students at Birkbeck, and a Cambridge chemist called Chris Abell.

Research in this company exploits fragment-based structural biology techniques for drug design. Last year the FDA approved the first drug to be designed at Astex, the kinase inhibitor ribociclib, as a treatment for advanced breast cancer in combination with letrozole. This drug is confidently expected to acquire the coveted 'blockbuster' status (over $1B in yearly sales) and there are others behind it in Astex' pipeline.

A later part of Blundell's talk described some of the work that underpinned and led to this success story: his own history, entwined with that of Birkbeck and of the discipline of structural biology. He joined the Department of Chemical Crystallography at the University of Oxford in 1964 to work for a PhD with Tiny Powell FRS before joining Dorothy Hodgkin's international team of crystallographers in 1967 to work on the structure of insulin. Hodgkin had obtained her first insulin crystals in 1934 but the structure was not finally solved for over thirty years; Blundell was lucky to be the first to present that structure at an international conference in 1969. At Birkbeck and beyond he became known for pioneering studies of HIV protease, showing its value as a drug target and laying the foundations for arguably the most successful class of drugs for AIDS. He is still an active researcher, and his group recently solved the structure of a DNA repair protein that holds the distinction of being the longest complete protein chain with a known structure. This protein, too, is a likely target for drugs against cancer. And he is bringing some of the techniques developed at Astex back into his academic lab, to design drugs for rare and neglected diseases including, respectively, cystic fibrosis and leprosy.

The next talk, by Birte Höcker from the University of Bayreuth in Germany, was badged with Blundell's in a section headed 'Computational and Chemical Biology'. Researchers based in Höcker's lab use computational methods to design protein sequences that will fold into particular structures. We have known for many years that protein sequence defines structure; that sequence and structure together define function, and that only a tiny proportion of possible sequences can form viable folds. Even the most complex of protein folds is built up from a number of smaller, stable units called domains, and these, in turn, are built up of fragments of structure that are smaller still.
Much of Höcker's talk focused on one of the commonest protein domain structures, named the 'TIM-barrel' after the first protein it was found in, the enzyme triose phosphate isomerase. This fold is actually a double barrel, with eight strands on the inside and eight helical segments on the outside of a cylindrical structure. It is also a symmetrical structure with two very similar halves, indicating that the TIM-barrel evolved through gene duplication. This putative ancestral 'half-barrel' looks very much like proteins that have a flavodoxin-like fold. Höcker's group combined parts of the genes for flavodoxin-like and TIM-barrel proteins; the protein that this produced folded into a stable structure that looked like a TIM barrel but had an extra strand. Computer-guided point mutations introduced into this strange protein, which Höcker termed a 'hopeful monster', produced an artificial protein with a standard TIM-barrel fold. This experiment shows that a TIM-barrel could have evolved from flavodoxin-like pieces, prompting Höcker to search for sequence-based evidence of such a relationship. Indeed, using recently developed algorithms, Höcker and her group could show that these seemingly different proteins are related. She also presented a collaborative experiment in which a TIM-barrel was built 'from scratch' for the first time: this has been a design goal for structural and computational biologists since 1986.



The next section, on Structural Biology, included three talks: one from an emeritus professor with a longer memory of structural biology at Birkbeck than even Blundell's; one from the ISMB's newest group leader; and the Rosalind Franklin lecture. The emeritus professor was Ken Holmes, who had studied for his PhD at Birkbeck in the 1950s with Rosalind Franklin herself (although formally supervised by J.D. Bernal) and spent almost all his distinguished career at the Max Planck Institute for Medical Research in Heidelberg, Germany. His fascinating talk focused on the topic he had studied for his Ph.D. and which he has maintained a strong interest in ever since: the structure of a plant virus known as tobacco mosaic virus. He showed many photos of the Georgian terrace in Bloomsbury that housed Birkbeck's biomolecular research lab in the 1950s and the equipment he

had used there, of the fibre diffraction patterns of the virus that he obtained and of models of the resulting helical structure. One of these was a polystyrene model, to scale but taller than Holmes himself, which was made for the 1958 World's Fair in Brussels. By the time this model was constructed, Franklin knew she had terminal cancer, and she never saw it exhibited; it was moved to the MRC Laboratory of Molecular Biology in Cambridge, where it stood in the foyer for many years. Holmes ended his talk with a moving description of the bravery and determination that Franklin had shown in her last months.

The next speaker, Philip Robinson, joined the ISMB as a group leader only a few months ago. His talk focused on his research as a postdoc in Roger Kornberg's lab at the University of Stanford, California, USA. Kornberg had been awarded the Nobel Prize in Chemistry in 2006 for studies of the molecular basis of DNA transcription in eukaryotic cells. His multidisciplinary team have made many advances in this field since then, mainly in the beginning of transcription, the process through which molecules of RNA are synthesised to match the DNA sequence of genes. This process is catalysed by an enzyme called RNA polymerase II (pol II for short) and it can only start when pol II is attached to the 'start point' of the DNA in complex with several other proteins. This whole assembly, known as the pre-initiation complex, includes pol II; several transcription factors; and a sub-complex with over 20 subunits that is collectively known as Mediator. Robinson's role in the project was to study the pre-initiation complex structure using electron microscopy, a task made harder by the fact that although the electron microscopy 'resolution revolution' was well underway, the Kornberg lab had not yet acquired one of the latest machines.

The Mediator complex is similar in all eukaryotes, where it is required for DNA transcription, and mutations in the human protein have been strongly linked to cancers. It can be subdivided into three modules, known as Head, Middle and Tail, and until recently the only structures of the intact complex have been very low-resolution 'blobs'. Robinson and his co-workers used electron microscopy, aided by mass spectrometry and chemical cross-linking, to obtain a structure for the whole pre-initiation complex that provided important insights into its mode of action. Very briefly, Mediator binds to the C-terminal domain (CTD) of one pol II subunit to bring the complex into contact with the DNA, and phosphorylation of the CTD after the whole complex has formed leads to Mediator release.

The annual Rosalind Franklin lecture has been held at Birkbeck since 2016; the lecture is part of Birkbeck's commitment to the Athena SWAN equality initiative and is always given by a woman scientist distinguished in one of the disciplines represented there. In a year when electron microscopy at Birkbeck has been so much to the fore, it was fitting that the lecture should be given by a proponent of this technique. Few women, with the possible exception of Birkbeck's own Helen Saibil, have achieved as much in electron microscopy as the 2018 Rosalind Franklin lecturer, Eva Nogales from the University of California in Berkeley, USA. Her lecture, which was an integral part of the ISMB symposium, dealt, like Robinson's, with the mechanisms through which human genes are expressed as RNA.

Nogales began her lecture with thanks: to the organisers for choosing her for this prestigious lecture, to Saibil for her inspiration, as one of the first women to gain a high profile in cryo-electron microscopy; and to Holmes, for his inspiring work on the structure of actin and actin filaments. She described some of the structures of protein complexes involved in the control of gene regulation that had been determined in her EM group. Polycomb repressive complex 2 (PRC2) is a large complex that acts as an enzyme, catalysing the addition of methyl groups to lysine residues in histones: proteins that bind to and package DNA in eukaryotic cells. This methylation causes the histone-DNA complexes, known as nucleosomes, to pack tightly, preventing access by RNA polymerase and thus turning off gene expression. PRC2 thus acts as a gene expression 'switch', and disrupting its function can lead to uncontrolled cell growth and multiplication; it is, therefore, a major drug design target in oncology. Nogales described the first EM structures of this complex, determined in 2012 before the so-called 'resolution revolution' in electron microscopy, as 'accurate but not very precise'. Researchers in her

group have now obtained structures at close to atomic resolution, showing that it can exist in two distinct, active conformational states. Differences include the position of one helical domain, which bends against the rest of the molecule in the so-called 'compact active' conformation and straightens away from it in the 'extended active' one. Its interactions with its co-factors, JARED and AEBP2, mimic the way that the complex binds to the 'tails' of the histones in order to carry out its catalytic activity.

The isolated PCR2 complex is an impressive enough structure, but Nogales went on to describe structures of the complex bound between a pair of nucleosomes, showing how the nucleosome substrate is positioned to bring the residues to be methylated into exactly the right position in the active site. She then turned to the other side of the process – RNA transcription and gene activation – and described some elegant structures of the 'pre-initiation complex' (PIC) that must form on the DNA before transcription can occur. She finished with largely unpublished work concerning the structure and dynamics of the human TFIID complex, which is required to nucleate the assembly of the PIC at the core promoter of every gene.

The second day of the symposium began with two lectures on the theme of Biophysics and Proteomics. The first to speak was Jody Puglisi from Stanford University, USA, and the focus of his talk was the second of the two main processes involved in gene expression: protein translation, which he described as a 'delicate dance' or 'ballet'. In all living organisms except viruses, the site of protein translation is the ribosome: a large 'molecular machine' composed of two subunits and with two binding sites. The RNA sequence is read and decoded by the small subunit; the large subunit contains the active site where bonds are formed between amino acids, adding units to the peptide chain. We now know that the subunits

rotate around each other after each new peptide bond is formed, and that it is this that moves the ribosome along the RNA so the next three bases can be decoded.

Puglisi described work in his lab to visualise and understand the dynamic molecular processes involved in protein synthesis, which involve thousands of atoms and take place over timescales ranging from picoseconds to seconds. Much of this involved the technique of single-molecule fluorescence resonance energy transfer (FRET), in which fluorescent dyes are bound to different molecules (or parts of a large molecule) and the distance between them monitored by measuring energy transfers between them. If one dye is bound to each subunit of a ribosome, each rotation following peptide bond formation can be detected as a change in fluorescence intensity. This process generally occurs smoothly; however, some ribosome-binding antibiotics act like grains of sand in a regular machine to disrupt the cycles and make the process less efficient. Modifying RNA codons can have a similar effect. In general, Puglisi concluded, we currently know more about the detailed mechanics of protein synthesis than about the biological role of each step: this is one system in which biophysics has overtaken biochemistry.

Like Puglisi, Petra Schwille from the Max Planck Institute of Biochemistry in Martinsried, Germany, studies living systems using single-molecule based techniques.

In her talk, she set out an ambitious challenge: to build a simple system with similar properties to a single cell from scratch. This is 'bottom-up synthetic biology' that aims to understand how life functions in its simplest form by building a living system from non-living parts. After discussing the minimal criteria for life, which include self-organisation, she described a minimal set of protein molecules in E. coli that control how the bacteria divide. The protein FtsZ self-assembles into a ring in the central region of a dividing bacterial cell. Other proteins are recruited onto this structure, which contracts when the cell divides,

eventually forming the new cell wall between the daughter cells. The position of the FtsZ ring is controlled by two further proteins, MinD (a membrane-bound ATPase) and MinE; these proteins work together as an oscillator to suppress ring formation anywhere but the exact centre of the cell, ensuring that cell division occurs correctly. This process can occur in vitro, and Schwille described progress towards the development of a system involving these proteins as part of a 'minimal [artificial] cell that can actually divide'.

The final session, under the theme of Biochemistry and Cell Biology, included three talks. The first of these, by Andrea Musacchio

from the Max Planck Institute of Molecular Physiology in Dortmund, Germany, continued with the theme of cell division but in eukaryotic rather than prokaryotic or 'minimal' cells. In mitosis – the name given to cell division in eukaryotes – each chromosome replicates to form two fused chromatids, which assemble on the mitotic spindle in the centre of the cell before the chromatids are pulled apart. "Like Siamese twins, chromatids are born united but have to be divided" he explained. The spindle is made up of microtubules, which attach to a disc-shaped structure at the centre of each chromatid, termed the kinetochore.

Musacchio described work in his lab and many others to determine the complex structure of the kinetochore. The inner kinetochore consists of a group of centromeric proteins (CENPs) that attach to a histone at the centromere known as CENP-A. This is memorably known as the 'chick-melon complex' from the distinguishing letters of its various CENPs: CENP-C, H, I, K, M, L and N. Its formation leads directly to an interaction between a nucleosome and the microtubule, and its stoichiometry is clearly defined. He described the reconstruction of a 21-subunit human centromere-kinetochore complex from purified components, centred on a single CENP-A nucleosome. The largest subunit, CENP-C, binds cooperatively to the nucleosome with CENP-N. Once the inner kinetochore has been assembled, further proteins are recruited to form the outer kinetochore, including 'antennae' that dock onto the kinetochore to bind microtubules. Musacchio presented detailed EM and X-ray structures of several subunits and groups of subunits in this complex, suggesting the hypothesis that the spindle checkpoint reads kinetochore structures to check that the chromatids are properly assembled.

Anthony Roberts, who joined the ISMB in 2014 after a post-doc at Harvard University, gave the second presentation from the 'home team'. His research focuses on the structure and mechanism of eukaryotic motor proteins, and his talk described recent research into one of these: dynein-2. This protein carries cargo molecules along microtubules from the tips of cilia into the body of the cells, but this raises the question of how it is initially targeted to the ciliary tip. Dynein-2 is an essential protein for the assembly and function of cilia, and mutations in it can cause serious skeletal and lung disorders. All dyneins belong to the AAA+ family of ATPases, which often assemble into rings of six identical subunits; dynein, uniquely in this family, has evolved to have a linear motor function.

Dynein-2 is a large multi-protein complex containing a dimer of motor domains. Research in Roberts' lab has shown that, when expressed as a monomer, the dynein-2 motor domain powers fast microtubule movement. However, as a dimer, the two motor domains inhibit one another. An EM reconstruction of the dimer showed the basis of this auto-inhibitory effect: the two motor domains stack against each other in a way that prevents them from producing movement. Based on additional functional experiments, Roberts presented a model in which auto-inhibition of dynein-2 facilitates its transport to the cilia tips by kinesin, another motor protein that moves along microtubules in the reverse direction.

The final presentation was given by another electron microscopist, Friedrich Forster from the University of Utrecht in the Netherlands. His group uses a variant of this technique, cryo-electron tomography, to study the molecular structures of protein complexes in their native state: that is, in living cells. This enables researchers to investigate the large and often transient complexes that are bound to cell and organelle membranes with the lipid membrane also

present. One key example of this is the ribosomes that are bound to the membranes of the endoplasmic reticulum (ER); proteins that are synthesised there enter the secretory pathway, and may end up bound to the cell membrane or secreted from the cell.

Ribosomes in the endoplasmic reticulum bind to pores in its membrane that are made up of a protein called sec61. Electron microscopy has been used to study the ribosome-sec61 complex for over twenty years. A combination of studies of isolated complexes using more traditional methods, and lower-resolution ones of membrane-bound complexes with cryo-electron tomography is yielding a complete picture of the structure and the machinery of membrane-associated protein synthesis. An associated RNA-binding protein called TRAP helps establish membrane protein topology. This protein is found in all eukaryotes, but it has a simpler structure in plants and yeast than in mammals, and studies of yeast TRAP are providing insights into the mechanism of the human protein.

Before the symposium was formally closed by Nicholas Keep, executive dean of the School of Science at Birkbeck, Waksman announced the winners of the Twitter competition and thanked all speakers and organisers, particularly the irreplaceable ISMB administrator, Andrew Service.